**Supporting Information**

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# *Ex-vivo* Immune-stimulating Activity of *Scutellaria baicalensis* and Its Major Flavonoids on Human Immune Cells

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## S1 : Materials and Methods

*S1. 1. Plant Material*

The roots of *Scutellaria baicalensis* Georgi were harvested in November 2017 and acquired from one-year-old plants grown by Prof. Jarmila Neugebauerová in the Experimental Garden of the Faculty of Horticulture, Mendel University in Brno (the Czech Republic). The voucher specimen (No. AL-748) was verified and deposited by Prof. Lubomír Opletal in the Department of Pharmaceutical Botany, Faculty of Pharmacy in Hradec Králové, Charles University (the Czech Republic). The drying process of the roots was performed at 40°C and the resulting dried material was grounded to a fine powder.

*S.1.2. Chemicals*

Solvents and chemicals were purchased from PENTA (the Czech Republic), VWR International (France), and Fisher Scientific (UK). Standards of baicalein (98% purity) and pokeweed mitogen (PWM) were obtained from Sigma-Aldrich (USA). The standard of baicalin (99% purity) was obtained from Extrasynthese (France). The standard wogonoside (98% purity) was acquired from Abcam (UK). Fluorescently marked antibodies were purchased from Exbio (the Czech Republic). MEM Alpha Medium was obtained from Life Technologies Europe (Netherlands).

*S.1.3. Preparation of the Lyophilized Extracts*

The aqueous extract (infusion) of SBR was prepared using 0.2 g of the dried powdered root extracted with 100 mL of boiling distilled water for 30 min. The resulting extract was lyophilized (Heto PowerDry LL3000, Thermo Electron, the Czech Republic) and stored in a fridge at 4°C. The ethanolic extract was prepared using 0.2 g of the dried powdered root extracted with 100 mL of absolute ethanol under sonication (Sonorex RK 100H, Bandelin electronics, Germany) for 30 min. The obtained extract was concentrated using a rotary evaporator (RVO 004, INGOS, the Czech Republic) at 40°C and further lyophilized. The extraction yield related to the dry weight of the powdered root mass was 30% for the aqueous extract and 13% for the ethanolic extract.

*S.1.4. Analysis of Immune Cell Activation*

Stock solutions of the two dried extracts and their main flavonoids baicalin, wogonoside, and baicalein (commercial standards) were prepared by dissolving 1 mg of samples in 1 mL of the mixture composed of 25 µL of dimethyl sulfoxide (DMSO) and 975 µL of MEM Alpha Medium. The obtained stock solutions were filtered through a nylon microfilter (0.2 µm, VWR International, USA) operating under aseptic conditions. Blood samples were drawn from three healthy human volunteers and collected into sodium heparinized tubes. For the analysis of immune cells, 100 µL of test samples was incubated with 100 µL of heparinized blood suspension in a sterile 96-well flat-bottomed plate at 37°C with 5% $CO_2$ for 24 h. The tests were carried out under ethics committee supervision (number: 10.11.2020) of the Faculty of Pharmacy, Charles University. In addition, the appropriate informed consent was obtained from all participants before blood samples were drawn. The final concentrations of ethanolic and aqueous SBR extracts in the assay media were in the range of 25–200 µg/mL, while the flavonoids were tested at 100 µg/mL [11]. Lectin from *Phytolacca americana* L. (pokeweed mitogen, PWM) was used as a positive control at a concentration of 10 µg/mL. The negative control was tested with DMSO (0.5%

**Table S1:** Content of flavonoids in ethanolic and aqueous extracts of *S. baicalensis* root

| Extracts | Flavonoid content (mg/g) | | |
|---|---|---|---|
| | **Baicalin** | **Wogonoside** | **Baicalein** |
| SBR ethanolic extract | 201.2 ± 0.6 | 47.7 ± 0.2 | 3.4 ± 0.1 |
| SBR aqueous extract | 150.4 ± 14.2 | 33.6 ± 3.0 | 4.6 ± 0.4 |

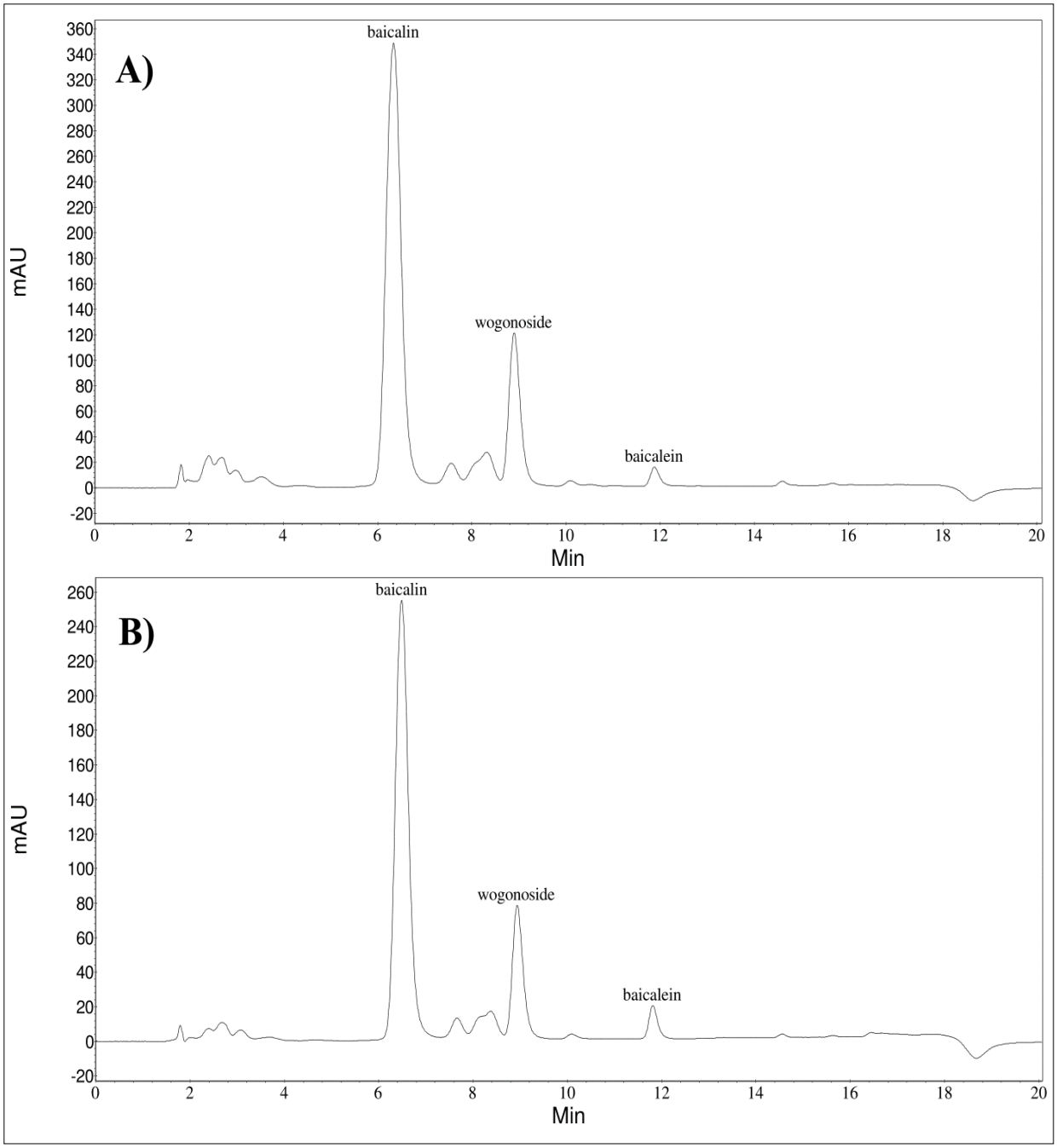


**Figure S1:** Representative HPLC chromatogram of the ethanolic (**A**) and aqueous (**B**) extracts of *S. baicalensis* root.